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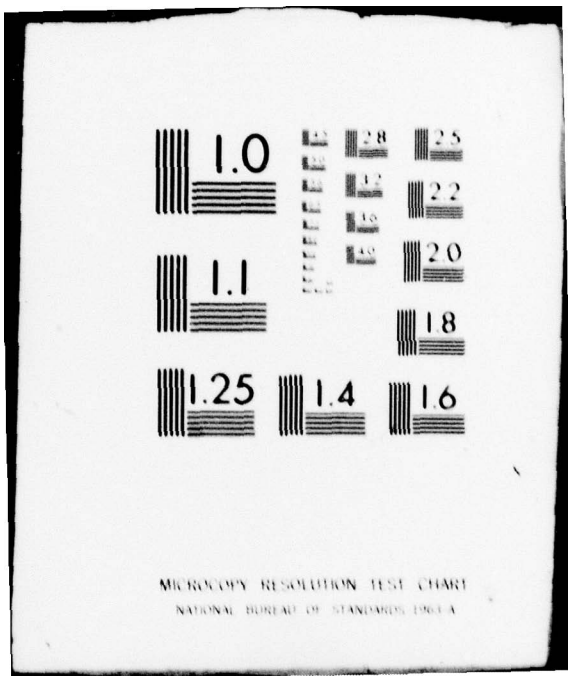
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CLASSES AND PROPERTIES OF HUMAN ANTIBODIES

Annual Report, Jun 74-Jul 75

July 1975

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by

Bernard B. Levine, M. D.

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Multiple injections of ovomucoid were given to mice with ongoing prolonged IgE antibody production to that antigen. Two inbred strains and antigen doses ranging from 0.05 to 5 μg each injection, given intradermally and subcutaneously, were used. Mice treated in this manner showed a marked diminution of the IgE antibody booster response as compared to controls. This decrease in booster response was antigen-specific. In addition, a protective effect from anaphylaxis was indicated. The mouse model continues to be a valuable tool for studies of certain IgE-mediated diseases.

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Progress Report July 31, 1975

CLASSES AND PROPERTIES OF HUMAN ANTIBODIES Bernard B. Levine, M.D.

Progress has been made on our studies on "hyposensitization" of mice with prolonged reagin production. We have published on the development of a mouse model for reagin hypersensitivity in man. The method consists of immunization with minute doses of protein in aluminum hydroxide gel. Crucial is a combination of antigen, dose and genetic strain of mouse. Thus both LAF_1 and $B_6D_2F_1$ hybrid mice develop prolonged reaginic antibody production after 2 injections of 0.2 μg ovomucoid 35 days apart. For LAF_1 mice serum reagin antibody is detected in the serum for the life of the animal. Also, in all ways tested to date, reagin production in the mouse model is similar to reagin production in spontaneously (by aeroallergens) sensitized man. Reaginic antibody in the mouse appears to be IgE-like.

The present work was done to study the effect of repeated injections of antigen in these sensitized mice upon reagin and IgG_1 antibody. The experiments were designed to mimic the "hyposensitization" therapy given human beings with IgE-mediated atopic diseases, as far as was possible.

Female mice, 9-11 weeks old, of the LAF_1 and $B_6D_2F_1$ strains were immunized with two intraperitoneal injections (35 days apart) of 0.2 μg purified ovomucoid plus 0.2 μg antigen E (from ragweed pollen extract) mixed with 0.5 ml PBS containing 2 mg of aluminum hydroxide gel. Sera were drawn at intervals and assayed for IgE (reagin) and IgG_1 antibodies by PCA in CFW female mice using 72 hour and 2 hour sensitization periods respectively. For IgE antibodies to ovomucoid either 150 μg or 1.5 μg of protein was used for elicitation. The lower dose gives titers two tubes (or greater) lower than the higher dose. The lower titers appear to represent the antibodies of relatively higher antigen binding avidities operationally, as they apparently capture antigen at lower concentrations. IgE antibodies specific for antigen E were assayed by using 6,000 PNU fresh short ragweed extract (reconstituted from lyphylized ragweed).

Antiovomucoid IgE antibodies were followed till the titers plateaued. One week after the second immunization (6th week of experiment) titers were 1/1280 to 1/640, and plateaued at 1:80 at the 20-30th week (for LAF_1). "Hyposensitization" injections were begun on the 31st week and continued to the 62nd week. Mice were divided into 5 groups of 6, a control (not injected) group and 4 injected groups. Injections were three times a week to reach a maximum on the 6th injection, then weekly for 4 weeks, then twice at maximum dosage a week for the remainder of the 31 weeks of injections. Maximum dosage was 0.5 or 5.0 μg subcut., or 0.05 or 0.5 μg I.D. Bloods were taken at intervals and assayed for antibodies. All animals were boosted (as in the two immunization injections) on the 73rd and again on the 84th week, 12 and 23 weeks after the "hyposensitization" series had ended. Sera were drawn after these boosts and assayed for IgG_1 and IgE antibodies.

The data are shown in Tables 1 and 2. The"hyposensitization series" of antigen injections resulted in a sharp booster of serum IgE and IgG_1 antibodies

to ovomucoid. As the antigen injections continued, titers of both IgG_1 and IgE antibodies fell. In the LAF_1 mice, titers of IgE antibodies appeared to fall more rapidly than did the titers of IgG_1 antibodies; in the $B_6D_2F_1$ mice the distinction was not as clear. At the end of the 31 weeks of hyposensitization, IgE antibodies titers were low (1:20 to 1:80) but significantly higher for the "hyposensitized" groups than for the control groups. However, a booster intraperitoneal injection of ovomucoid (9 or 11 weeks after the end of the course of hyposensitization) resulted in a sharp rise of IgE antibodies in the control groups, while it caused either no (or a much lower) rise in IgE antibodies in the "hyposensitized" groups. Similar booster effects were noted for IgE antibodies. Thus after the booster injection (week 73 or 75) titers of IgE antibody specific for ovomucoid were significantly higher in the control groups, than in the "hyposensitized" groups. Eleven weeks after, another booster injection was given the control and "hyposensitized" groups with similar results. At the end of this experiment (week 85 or 87), titers of IgE antibody specific for ovomucoid were considerably higher for the control than for the hyposensitized groups. These differences were specific; there were no clear differences between control and hyposensitized groups in antibodies specific for ragweed extract (antigen E).

Three weeks after the 2nd booster injection (weeks 87 or 89) the mice were injected with 10 μg ovovmucoid to determine whether the "hyposensitized" groups had been protected against anaphylaxis. The 10 μg dose was used as in a preliminary experiment on 2 controls, 1 μg i.v. did not cause death. These two mice were not used in subsequent experiments. Table III shows that mice in the "hyposensitized" groups were less frequently killed by anaphylaxis than were mice in the control groups.

These data are similar to those obtained in studies on the immunological changes to "hyposensitization" injections in humans with ragweed hay fever. It provides increasing support for our notion that this is a useful experimental animal model for IgE-mediated allergic diseases in man.

We plan to continue with experiments to elucidate immunological mechanisms underlying these observations, and to test different therapies in this model.

TABLE I: IgG_1 and IgE antibody titers in "hyposensitized" and control LAF_1 mice[a]

Week of Experiment	Control Group[b]			Group I (5 μg ovomucoid S.C.)			Group II (0.5μg ovomucoid I.D.)			Group III (0.5μg ovomucoid S.C.)			Group IV (0.05μg ovomucoid I.D.)		
	IgG_1 ovo-mucoid[c,d]	IgE ovo-mucoid[c,d]	IgE Rag-weed[c,d]	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed
33	1:80	1:80	-	1:5120	1:2560	-	1:2560	1:320	-	1:2560	1:2560	-	1:2560	1:640	-
35	1:80	1:80	-	1:1280	1:1280	-	1:1280	1:320	-	1:1280	1:1280	-	1:1280	1:320	-
39	1:20	1:80	-	1:1280	1:640	-	1:640	1:320	-	1:1280	1:640	-	1:320	1:640	-
45	< 1:20	1:80	-	1:1280	1:320	-	1:1280	1:160	-	1:640	1:160	-	1:320	1:160	-
49	1:5	1:80	-	1:1280	1:320	-	1:1280	1:160	-	1:640	1:160	-	1:160	1:160	-
53	1:5	1:80	1:80	1:1280	1:80	< 1:20	1:1280	1:160	< 1:20	1:640	1:80	< 1:20	1:160	1:40	< 1:20
57	1:5	1:20	1:20	1:1280	1:80	< 1:5	1:640	1:80	1:5	1:320	1:80	< 1:5	1:320	1:80	1:5
62		1:20			1:80			1:80			1:80			1:80	
70	< 1:5	1:20	1:5	1:1280	1:80	< 1:5	1:160	1:20	< 1:5	1:320	1:80	1:5	1:320	1:80	1:5
73	Third Immunization														
74	1:80	1:1930	1:50	1:520	1:80	1:320	1:213	1:80	1:290	1:1280	1:173	1:90	1:480	1:320	1:260
78	1:160	1:640	1:40	1:640	1:80	1:160	1:640	1:40	1:80	1:1280	1:80	1:40	1:640	1:40	1:40
84	1:160	1:640	1:40	1:1280	1:40	1:80	1:640	1:40	1:40	1:640	1:160	1:40	1:640	1:160	1:40
84	Fourth Immunization														
85	1:640	1:2560	1:40	1:1280	1:80	1:320	1:1280	1:80	1:80	1:1280	1:80	1:80	1:1280	1:80	1:40

a- Hyposensitization with ovomucoid began at 31st week of experiment - with 5 increments of dosage over a 9 day period - then the maximum dose weekly for four weeks then - given twice weekly until the 62nd week of the experiment.

b- Groups of 7-8 mice each were made from a population whose pooled sera IgG and IgE antibody titers were 1:80 and 1:160 for ovomucoid, and 1:80 for Ragweed at week 27 of experiment.

c- IgG and IgE titers were obtained by PCA assay in 6-8 week old CFW female mice, IgG, 2 hour sensitization period; IgE 72 hour sensitization period. Sera were drawn 3 or 4 days after last antigen injection.

d- Antigen challenge dose was 150 μg i.v. for ovomucoid and 6000 pnu i.v. for Ragweed.

TABLE II: IgG_1 and IgE antibody titers in "hyposensitized" and control B_6D_2 mice[a]

Week of Experiment	Control Group[b]			Group I (5 μg ovomucoid S.C.)			Group II (0.5μg ovomucoid I.D.)			Group III (0.5μg ovomucoid S.C)			Group IV (0.05μg ovomucoid I.D.)		
	IgG_1 ovo-mucoid[c,d]	IgE ovo-mucoid[c,d]	IgE Rag-weed[c,d]	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed	IgG_1 ovo-mucoid	IgE ovo-mucoid	IgE Rag-weed
35	< 1:20	1:20	-	1:2560	1:1280	-	1:320	1:320	-	1:640	1:320	-	1:160	1:320	-
37	< 1:20	< 1:20	-	1:640	1:320	-	1:640	1:320	-	1:320	1:160	-	1:80	1:80	-
41	< 1:20	< 1:20	-	1:160	1:80	-	1:80	1:80	-	1:80	1:80	-	1:40	1:40	-
47	< 1:5	< 1:5	-	1:80	1:160	-	1:80	1:160	-	1:80	1:80	-	1:80	1:80	-
51	< 1:5	< 1:5	-	1:80	1:160	-	1:80	1:80	-	1:80	1:80	-	1:80	1:20	-
64	< 1:5	< 1:5	< 1:5		1:80	1:5		1:20	1:5		1:20	1:5		1:20	1:5
70	< 1:5	< 1:5	< 1:5	1:20	1:80	< 1:5	1:5	1:20	< 1:5	1:20	1:20	< 1:5	1:20	1:20	< 1:5
75	Third Immunization														
76	1:160	1:160	1:160	1:40	1:40	1:160	1:10	1:10	1:40	1:40	1:20	1:160	1:40	1:40	1:160
80	1:20	1:80	1:320	1:80	1:40	1:80	1:20	1:20	1:80	1:80	1:80	1:20	1:80	1:80	1:80
86	1:20	1:80	1:40	1:20	1:40	1:10	< 1:10	1:10	1:10	1:40	1:40	< 1:10	1:20	1:40	1:10
86	Fourth Immunization														
87	1:320	1:640	1:320	1:160	1:160	1:40	1:40	1:40	1:40	1:40	1:40	1:40	1:80	1:40	1:40

a- Hyposensitization with ovomucoid began at 33rd week of experiment with 5 increments of dosage over a 9 day period - The maximum dose given weekly for four weeks then twice weekly to 64th week of experiment.

b- Groups of 7-8 mice each were made from total population whose pooled sera IgG_1 and IgE antibody titers were < 1:20 and 1:40 for ovomucoid, 1:40 for Ragweed at week 27 of experiment.

c- IgG_1 and IgE titers were obtained by PCA assay in 6-8 week old CFW female mice, IgG_1 2 hour sensitization period; IgE 72 hour sensitization period. Sera were drawn 3 or 4 days after last antigen injection.

d- Antigen challenge dose was 150 μg i.v. for ovomucoid and 6000 pnu i.v. for Ragweed.

TABLE III: Anaphylactic Mortality*

	Anaphylactic deaths	Average time to death
LAF_1 groups		
Controls	4/4	18 min
Group I (5 μg S.C.)	3/4	46
Group II (0.5 μg I.D.)	1/3	44
Group III (0.5 μg S.C.)	2/3	25
Group IV (0.05 μg I.D.)	3/3	23
$B_6D_2F_1$ groups		
Controls	4/7	41 min
Group I	1/4	76 min
Group II	1/4	> 6 hours
Group III	0/4	-
Group IV	1/4	43 min

*To 10 μg ovomucoid injected I.V.

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